33. Engineering Crassulacean Acid Metabolism (CAM) to Improve Water-use Efficiency of Bioenergy Feedstocks

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Project Goals: The long-term goal of this project is to enhance the water-use efficiency (WUE) and adaptability to hotter, drier climates of species that normally perform C3 photosynthesis by introducing crassulacean acid metabolism (CAM). Photosynthetic performance and WUE will be enhanced in Arabidopsis and Populus by: 1) defining the genetic basis of key CAM modules in both eudicot and monocot CAM species, 2) characterizing the regulation of 'carboxylation', 'decarboxylation', and 'inverse stomatal control' gene modules of CAM using a wide variety of functional genomic approaches including loss-of-function studies in model CAM species, 3) deploying advanced genome engineering technologies to enable transfer of fully functional CAM modules into C3 species and 4) analyzing the effects of these transgenic modules on 'stomatal control', CO2 assimilation and transpiration rates, biomass yield, and WUE in Arabidopsis and Populus.

A changing global climate is predicted to lead to declines in agricultural and agroforestry productivity due to increasing water vapor-pressure deficits. One potential approach to improve the sustainability of biofuels feedstocks will be to move the water-wise photosynthetic machinery of CAM into C3 food and bioenergy crops (1). Crassulacean acid metabolism (CAM) is an adaptation that reduces water loss through stomata by shifting all or part of CO2 fixation from the daytime to the nighttime, which results in improved water-use efficiency (WUE) by reductions in evapotranspiration. Introducing the CAM pathway into C3 plants is expected to confer improved WUE in order to allow plants to withstand long episodes of drought or perhaps to expand crop production into semi-arid regions. An essential prerequisite for engineered CAM is to characterize and functionally analyze the leaf anatomy and minimal set of genes and proteins required for its efficient operation.

Introducing the CAM pathway into C3 plants such as Arabidopsis and Populus will likely require several important tasks: (i) the proper temporal and mesophyll-specific expression patterns of multiple C4 enzymes, their regulatory protein kinases/phosphatases, and related transporters in order to reconstitute the CAM photosynthetic machinery; (ii) an increased mesophyll cell size in order to store malate in the vacuoles of mesophyll cells and limit CO2 diffusion; (iii) an effective multiple gene assembly strategy to effectively express a large number of expression cassettes to build insulate genetic circuits; and (iv) strategies to overcome heterochromatin-mediated gene silencing and reduced mRNA expression of adjacent genes within cis-acting components of gene cassettes to prevent transgene silencing and ensure stable expression of CAM pathway enzymes.
To date, progress has been made in the identification CAM-specific genes that constitute the functional

'carboxylation' and 'decarboxylation' modules of CAM, the identification and selection of promoter regions that are predicted to drive the appropriate temporal (circadianclock controlled), and mesophyll-specific expression patterns required for CAM, as well as drought-induced promoters for engineering the facultative engagement of CAM, and a GUS/LUC reporter system to validate these predicted expression patterns. Furthermore, increased leaf succulence, an anatomical trait associated with CAM that limits CO2 diffusion out of the leaf, has been engineered in Arabidopsis. Lastly, insulated genetic circuits are being created using a biologically inactive, unique nucleotide sequence (UNS)-guided Gibson isothermal assembly strategy (2, 3) using the gypsy insulator (4) to ensure the optimal expression of CAM genes cassettes by preventing transgene silencing in Arabidopsis. Future efforts will focus on moving the completed genetic circuits (e.g., 'carboxylation' and 'decarboxylation' modules) into Arabidopsis, and testing transgenic plants for CO2 assimilation and transpiration rates, WUE, and biomass yield. Once proof-of-concept designs are functionally validated in Arabidopsis, synthetic 'carboxylation' and 'decarboxylation' modules will be moved to Populus for further functional testing.

References

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